

We claim:

Sub 9
5 1. A substantially purified sortase-transamidase enzyme from a Gram-positive bacterium, the enzyme catalyzing a reaction that covalently cross-links the carboxyl terminus of a protein having a sorting signal to the peptidoglycan of a Gram-positive bacterium, the sorting signal having a motif of LPX₃X₄G therein, wherein sorting occurs by cleavage between the fourth and fifth residues of the LPX₃X₄G motif. ✓

10 2. The substantially purified sortase-transamidase enzyme of claim 1 wherein the Gram-positive bacterium is a species selected from the group consisting of *S. aureus*, *S. sobrinus*, *B. subtilis*, *E. faecalis*, *S. pyogenes*, *S. pneumoniae*, and *L. monocytogenes*.

15 3. The substantially purified sortase-transamidase enzyme of claim 2 wherein the Gram-positive bacterium is *Staphylococcus aureus*.

20 4. The substantially purified sortase-transamidase enzyme of claim 1 wherein the enzyme has a molecular weight of about 23,539 daltons.

25 5. The substantially purified sortase-transamidase enzyme of claim 4 wherein the sorting signal further comprises: (2) a substantially hydrophobic domain of at least 31 amino acids carboxyl to the motif; and (3) a charged tail region with at least two positively charged residues carboxyl to the substantially hydrophobic domain, at least one of the two positively charged residues being arginine, the two positively charged residues being located at residues 31-33 from the motif, wherein X₃ is any of the twenty naturally-occurring L-amino acids and X₄ is selected from the group consisting of alanine, serine, and threonine.

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6. The enzyme of claim 1 wherein the enzyme includes therein an amino acid sequence selected from the group consisting of: (1) M-K-K-W-T-N-R-L-M-T-I-A-G-V-V-L-I-L-V-A-A-Y-L-F-A-K-P-H-I-D-N-Y-L-H-D-K-D-K-D-E-K-I-E-Q-Y-D-K-N-V-K-E-Q-A-S-K-D-K-K-Q-Q-A-K-P-Q-I-P-K-D-K-S-K-V-A-G-Y-I-E-I-P-D-A-D-I-K-E-P-V-Y-P-G-P-A-T-P-E-Q-L-N-R-G-V-S-F-A-E-E-N-E-S-L-D-D-Q-N-I-S-I-A-G-H-T-F-I-D-R-P-N-Y-Q-F-T-N-L-K-A-A-K-K-G-S-M-V-Y-F-K-V-G-N-E-T-R-K-Y-K-M-T-S-I-R-D-V-K-P-T-D-V-G-V-L-D-E-Q-K-G-K-D-K-Q-L-T-L-I-T-C-D-D-Y-N-E-K-T-G-V-W-E-K-R-K-I-F-V-A-T-E-V-K (SEQ ID NO: 3); and (2) sequences incorporating one or more conservative amino acid substitutions in SEQ ID NO:3, wherein the conservative amino acid substitutions are any of the following: (1) any of isoleucine, leucine, and valine for any other of these amino acids; (2) aspartic acid for glutamic acid and vice versa; (3) glutamine for asparagine and vice versa; and (4) serine for threonine and vice versa.

7. The enzyme of claim 6 wherein the amino acid sequence is M-K-K-W-T-N-R-L-M-T-I-A-G-V-V-L-I-L-V-A-A-Y-L-F-A-K-P-H-I-D-N-Y-L-H-D-K-D-K-D-E-K-I-E-Q-Y-D-K-N-V-K-E-Q-A-S-K-D-K-K-Q-Q-A-K-P-Q-I-P-K-D-K-S-K-V-A-G-Y-I-E-I-P-D-A-D-I-K-E-P-V-Y-P-G-P-A-T-P-E-Q-L-N-R-G-V-S-F-A-E-E-N-E-S-L-D-D-Q-N-I-S-I-A-G-H-T-F-I-D-R-P-N-Y-Q-F-T-N-L-K-A-A-K-K-G-S-M-V-Y-F-K-V-G-N-E-T-R-K-Y-K-M-T-S-I-R-D-V-K-P-T-D-V-G-V-L-D-E-Q-K-G-K-D-K-Q-L-T-L-I-T-C-D-D-Y-N-E-K-T-G-V-W-E-K-R-K-I-F-V-A-T-E-V-K (SEQ ID NO: 3).

8. A nucleic acid sequence encoding the enzyme of claim 6.

9. A nucleic acid sequence encoding the enzyme of claim 7.

10. A nucleic acid sequence encoding a substantially purified sortase-transamidase enzyme from a Gram-positive bacterium, the enzyme having a molecular weight of about 23,539 daltons and catalyzing a reaction that covalently cross-links the carboxyl terminus of a protein having a sorting signal to the peptidoglycan of a Gram-positive bacterium, the sorting signal having: (1) a motif of LPX₃X₄G therein; (2) a substantially hydrophobic domain of at least 31 amino acids carboxyl to the motif; and (3) a charged tail region with at least two positively charged residues carboxyl to the substantially hydrophobic domain, at least one of the two positively charged residues being arginine, the two positively charged residues being located at residues 31-33 from the motif, wherein X₃ is any of the twenty naturally-occurring L-amino acids and X₄ is selected from the group consisting of alanine, serine, and threonine, and wherein sorting occurs by cleavage between the fourth and fifth residues of the LPX₃X₄G motif, wherein the nucleic acid sequence includes therein a sequence selected from the group consisting of: (1) ATGAAAAAATGGACAAATCGATTAATGACAATCGCTGGTGTGGTACTTATCCTAGTG GCAGCATATTTGTTTGCTAAACCACATATCGATAATTATCTTCACGATAAAGATAAA GATGAAAAGATTGAACAATATGATAAAAATGTAAAAGAACAGGCGAGTAAAGATA AAAAGCAGCAAGCTAAACCTCAAATTCGAAAGATAAATCGAAAGTGGCAGGCTAT ATTGAAATTCCAGATGCTGATATTAAGAACCAGTATATCCAGGACCAGCAACACCT GAACAATTAAATAGAGGTGTAAGCTTTGCAGAAGAAAATGAATCACTAGATGATCA AAATATTTCAATTGCAGGACACACTTTCATTGACCGTCCGAACCTATCAATTTACAAA TCTTAAAGCAGCCAAAAAAGGTAGTATGGTGTACTTTAAAGTTGGTAATGAAACAC GTAAGTATAAAATGACAAGTATAAGAGATGTTAAGCCTACAGATGTAGGAGTTCTA GATGAACAAAAAGGTAAAGATAAACAATTAACATTAATTACTTGTGATGATTACAA TGAAAAGACAGGCGTTTGGGAAAAACGTAAAATCTTTGTAGCTACAGAAGTCAAAT AA (SEQ ID NO: 2); or (2) a sequence complementary to SEQ ID NO: 2.

11. A nucleic acid sequence encoding a substantially purified sortase-transamidase enzyme from a Gram-positive bacterium, the enzyme having a molecular weight of about 23,539 daltons and catalyzing a reaction that covalently cross-links the carboxyl terminus of a protein having a sorting signal to the peptidoglycan of a Gram-positive bacterium, the sorting signal having (1) a motif of LPX₃X₄G therein; (2) a substantially hydrophobic domain of at least 31 amino acids carboxyl to the motif; and (3) a charged tail region with at least two positively charged residues carboxyl to the substantially hydrophobic domain, at least one of the two positively charged residues being arginine, the two positively charged residues being located at residues 31-33 from the motif, wherein X₃ is any of the twenty naturally-occurring L-amino acids and X₄ is selected from the group consisting of alanine, serine, and threonine, and wherein sorting occurs by cleavage between the fourth and fifth residues of the LPX₃X₄G motif, wherein the nucleic acid sequence hybridizes with a sequence selected from the group consisting of: (1) ATGAAAAAATGGACAAATCGATTAATGACAATCGCTGGTGTGGTACTTATCCTAGTGGCAGCATATTTGTTTGCTAAACCACATATCGATAATTATCTTCACGATAAAGATAAAGATGATGAAAAGATTGAACAATATGATAAAAATGTAAAAGAACAGGCGAGTAAAGATAAAAAGCAGCAAGCTAAACCTCAAATTCCGAAAGATAAATCGAAAGTGGCAGGCTATATTGAAATTCCAGATGCTGATATTAAAGAACCAGTATATCCAGGACCAGCAACACCTGAACAATTAAATAGAGGTGTAAGCTTTGCAGAAGAAAATGAATCACTAGATGATCAAAATATTTCAATTGCAGGACACACTTTCATTGACCGTCCGAACCTATCAATTTACAAATCTTAAAGCAGCCAAAAAAGGTAGTATGGTGTACTTTAAAGTTGGTAATGAAACACGTAAGTATAAAATGACAAAGTATAAGAGATGTAAAGCCTACAGATGTAGGAGTTCTAGATGAACAAAAAGGTAAAGATAAACAATTAACATTAATTACTTGTGATGATTACAAATGAAAAGACAGGCGTTTGGGAAAAACGTAAAATCTTTGTAGCTACAGAAGTCAAATAA (SEQ ID NO: 2) or (2) a sequence complementary to SEQ ID NO: 2, with no greater than about a 15% mismatch under stringent conditions.

12. The nucleic acid sequence of claim 11 wherein the mismatch is no greater than about 5%.

506 417 13. The nucleic acid sequence of claim 11 wherein the mismatch is no greater than about 2%.

5 14. A vector comprising the nucleic acid sequence of claim 8 operatively linked to at least one control sequence that controls the expression or regulation of the nucleic acid sequence.

10 15. A vector comprising the nucleic acid sequence of claim 9 operatively linked to at least one control sequence that controls the expression or regulation of the nucleic acid sequence.

15 16. A vector comprising the nucleic acid sequence of claim 10 operatively linked to at least one control sequence that controls the expression or regulation of the nucleic acid sequence.

20 17. A vector comprising the nucleic acid sequence of claim 11 operatively linked to at least one control sequence that controls the expression or regulation of the nucleic acid sequence.

18. A host cell transfected with the vector of claim 14.

19. A host cell transfected with the vector of claim 15.

25 20. A host cell transfected with the vector of claim 16.

21. A host cell transfected with the vector of claim 17.

22. A method for producing a substantially purified sortase-transamidase enzyme ✓
comprising the steps of:

(a) culturing the host cell of claim 18 under conditions in which the host cell
expresses the encoded sortase-transamidase enzyme; and

(b) purifying the expressed enzyme to produce substantially purified sortase-
transamidase enzyme.

23. A method for producing a substantially purified sortase-transamidase enzyme
comprising the steps of:

(a) culturing the host cell of claim 19 under conditions in which the host cell
expresses the encoded sortase-transamidase enzyme; and

(b) purifying the expressed enzyme to produce substantially purified sortase-
transamidase enzyme.

24. A method for producing a substantially purified sortase-transamidase enzyme
comprising the steps of:

(a) culturing the host cell of claim 20 under conditions in which the host cell
expresses the encoded sortase-transamidase enzyme; and

(b) purifying the expressed enzyme to produce substantially purified sortase-
transamidase enzyme.

25. A method for producing a substantially purified sortase-transamidase enzyme
comprising the steps of:

(a) culturing the host cell of claim 21 under conditions in which the host cell
expresses the encoded sortase-transamidase enzyme; and

(b) purifying the expressed enzyme to produce substantially purified sortase-
transamidase enzyme.

26. Substantially purified sortase-transamidase enzyme produced by the process of claim 22.

5 27. Substantially purified sortase-transamidase enzyme produced by the process of claim 23.

28. Substantially purified sortase-transamidase enzyme produced by the process of claim 24.

10 29. Substantially purified sortase-transamidase enzyme produced by the process of claim 25.

30. A method for screening a compound for anti-sortase-transamidase activity comprising the steps of:

15 (a) providing the substantially purified sortase-transamidase enzyme of claim 1;

(b) performing an assay for sortase-transamidase in the presence and in the absence of the compound; and

(c) comparing the activity of the sortase-transamidase enzyme in the presence and in the absence of the compound to screen the compound for sortase-transamidase activity.

20 31. A method for screening a compound for anti-sortase-transamidase activity comprising the steps of:

(a) providing the substantially purified sortase-transamidase enzyme of claim 3;

25 (b) performing an assay for sortase-transamidase in the presence and in the absence of the compound; and

(c) comparing the activity of the sortase-transamidase enzyme in the presence and in the absence of the compound to screen the compound for sortase-transamidase activity.

32. A method for screening a compound for anti-sortase-transamidase activity comprising the steps of:

- 5 (a) providing the substantially purified sortase-transamidase enzyme of claim 26;
(b) performing an assay for sortase-transamidase in the presence and in the absence of the compound; and
(c) comparing the activity of the sortase-transamidase enzyme in the presence and in the absence of the compound to screen the compound for sortase-transamidase activity.

33. A method for screening a compound for anti-sortase-transamidase activity comprising the steps of:

- 10 (a) providing the substantially purified sortase-transamidase enzyme of claim 27;
(b) performing an assay for sortase-transamidase in the presence and in the absence of the compound; and
(c) comparing the activity of the sortase-transamidase enzyme in the presence and in the absence of the compound to screen the compound for sortase-transamidase activity.

34. A method for screening a compound for anti-sortase-transamidase activity comprising the steps of:

- 15 (a) providing the substantially purified sortase-transamidase enzyme of claim 28;
20 (b) performing an assay for sortase-transamidase in the presence and in the absence of the compound; and
(c) comparing the activity of the sortase-transamidase enzyme in the presence and in the absence of the compound to screen the compound for sortase-transamidase activity.

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35. A method for screening a compound for anti-sortase-transamidase activity comprising the steps of:

- (a) providing the substantially purified sortase-transamidase enzyme of claim 29;
- (b) performing an assay for sortase-transamidase in the presence and in the absence of the compound; and
- (c) comparing the activity of the sortase-transamidase enzyme in the presence and in the absence of the compound to screen the compound for sortase-transamidase activity.

36. A method for screening a compound for anti-sortase-transamidase activity comprising the steps of:

- (a) providing an active fraction of sortase-transamidase enzyme from a Gram-positive bacterium;
- (b) performing an assay for sortase-transamidase in the presence and in the absence of the compound; and
- (c) comparing the activity of the sortase-transamidase enzyme in the presence and in the absence of the compound to screen the compound for sortase-transamidase activity.

37. The method of claim 36 wherein the active fraction of sortase-transamidase enzyme is a particulate fraction from *Staphylococcus aureus*.

38. The method of claim 36 wherein the assay for sortase-transamidase enzyme is performed by monitoring the capture of a soluble peptide that is a substrate for the enzyme by its interaction with an affinity resin.

39. The method of claim 38 wherein the soluble peptide includes a sequence of at least six histidine residues and the affinity resin contains nickel.

40. The method of claim 38 wherein the soluble peptide includes the active site of glutathione S-transferase and the affinity resin contains glutathione.

5 41. The method of claim 38 wherein the soluble peptide includes the active site of streptavidin and the affinity resin contains biotin.

42. The method of claim 38 wherein the soluble peptide includes the active site of maltose binding protein and the affinity resin contains amylose.

10 43. An antibody specifically binding the substantially purified sortase-transamidase enzyme of claim 1.

15 44. An antibody specifically binding the substantially purified sortase-transamidase enzyme of claim 3.

45. An antibody specifically binding the substantially purified sortase-transamidase enzyme of claim 26.

20 46. An antibody specifically binding the substantially purified sortase-transamidase enzyme of claim 27.

47. An antibody specifically binding the substantially purified sortase-transamidase enzyme of claim 28.

25 48. An antibody specifically binding the substantially purified sortase-transamidase enzyme of claim 29.

54 49. A protein molecule comprising the substantially purified sortase-transamidase enzyme of claim 1 extended at its carboxyl-terminus with a sufficient number of histidine residues to allow specific binding of the protein molecule to a nickel-sepharose column through the histidine residues added at the carboxyl-terminus.

5 50. A protein molecule comprising the substantially purified sortase-transamidase enzyme of claim 3 extended at its carboxyl-terminus with a sufficient number of histidine residues to allow specific binding of the protein molecule to a nickel-sepharose column through the histidine residues added at the carboxyl-terminus.

10 51. A protein molecule comprising the substantially purified sortase-transamidase enzyme of claim 26 extended at its carboxyl-terminus with a sufficient number of histidine residues to allow specific binding of the protein molecule to a nickel-sepharose column.

15 52. A protein molecule comprising the substantially purified sortase-transamidase enzyme of claim 27 extended at its carboxyl-terminus with a sufficient number of histidine residues to allow specific binding of the protein molecule to a nickel-sepharose column.

20 53. A protein molecule comprising the substantially purified sortase-transamidase enzyme of claim 28 extended at its carboxyl-terminus with a sufficient number of histidine residues to allow specific binding of the protein molecule to a nickel-sepharose column.

25 54. A protein molecule comprising the substantially purified sortase-transamidase enzyme of claim 29 extended at its carboxyl-terminus with a sufficient number of histidine residues to allow specific binding of the protein molecule to a nickel-sepharose column.

55. A method for displaying a polypeptide on the surface of a Gram-positive bacterium comprising the steps of:

(a) expressing a polypeptide having a sorting signal at its carboxy-terminal end, the sorting signal having: (1) a motif of LPX_3X_4G therein; (2) a substantially hydrophobic domain of at least 31 amino acids carboxyl to the motif; and (3) a charged tail region with at least two positively charged residues carboxyl to the substantially hydrophobic domain, at least one of the two positively charged residues being arginine, the two positively charged residues being located at residues 31-33 from the motif, wherein X_3 is any of the twenty naturally-occurring L-amino acids and X_4 is selected from the group consisting of alanine, serine, and threonine;

(b) forming a reaction mixture including: (i) the expressed polypeptide; (ii) the substantially purified sortase-transamidase of claim 1; and (iii) a Gram-positive bacterium having a peptidoglycan to which the sortase-transamidase can link the polypeptide; and

(c) allowing the sortase-transamidase to catalyze a reaction that cleaves the polypeptide within the LPX_3X_4 motif of the sorting signal and covalently cross-links the amino-terminal portion of the cleaved polypeptide to the peptidoglycan to display the polypeptide on the surface of the Gram-positive bacterium.

56. A method for displaying a polypeptide on the surface of a Gram-positive bacterium comprising the steps of:

(a) expressing a polypeptide having a sorting signal at its carboxy-terminal end, the sorting signal having: (1) a motif of LPX₃X₄G therein; (2) a substantially hydrophobic domain of at least 31 amino acids carboxyl to the motif; and (3) a charged tail region with at least two positively charged residues carboxyl to the substantially hydrophobic domain, at least one of the two positively charged residues being arginine, the two positively charged residues being located at residues 31-33 from the motif, wherein X₃ is any of the twenty naturally-occurring L-amino acids and X₄ is selected from the group consisting of alanine, serine, and threonine;

(b) forming a reaction mixture including: (i) the expressed polypeptide; (ii) the substantially purified sortase-transamidase of claim 3; and (iii) a Gram-positive bacterium having a peptidoglycan to which the sortase-transamidase can link the polypeptide; and

(c) allowing the sortase-transamidase to catalyze a reaction that cleaves the polypeptide within the LPX₃X₄G motif of the sorting signal and covalently cross-links the amino-terminal portion of the cleaved polypeptide to the peptidoglycan to display the polypeptide on the surface of the Gram-positive bacterium.

57. A method for displaying a polypeptide on the surface of a Gram-positive bacterium comprising the steps of:

(a) expressing a polypeptide having a sorting signal at its carboxy-terminal end, the sorting signal having: (1) a motif of LPX_3X_4G therein; (2) a substantially hydrophobic domain of at least 31 amino acids carboxyl to the motif; and (3) a charged tail region with at least two positively charged residues carboxyl to the substantially hydrophobic domain, at least one of the two positively charged residues being arginine, the two positively charged residues being located at residues 31-33 from the motif, wherein X_3 is any of the twenty naturally-occurring L-amino acids and X_4 is selected from the group consisting of alanine, serine, and threonine;

(b) forming a reaction mixture including: (i) the expressed polypeptide; (ii) the substantially purified sortase-transamidase enzyme of claim 26; and (iii) a Gram-positive bacterium having a peptidoglycan to which the sortase-transamidase can link the polypeptide; and

(c) allowing the sortase-transamidase to catalyze a reaction that cleaves the polypeptide within the LPX_3X_4G motif of the sorting signal and covalently cross-links the amino-terminal portion of the cleaved polypeptide to the peptidoglycan to display the polypeptide on the surface of the Gram-positive bacterium.

58. A method for displaying a polypeptide on the surface of a Gram-positive bacterium comprising the steps of:

5 (a) expressing a polypeptide having a sorting signal at its carboxy-terminal end, the sorting signal having: (1) a motif of LPX₃X₄G therein; (2) a substantially hydrophobic domain of at least 31 amino acids carboxyl to the motif; and (3) a charged tail region with at least two positively charged residues carboxyl to the substantially hydrophobic domain, at least one of the two positively charged residues being arginine, the two positively charged residues being located at residues 31-33 from the motif, wherein X₃ is any of the twenty naturally-occurring L-amino acids and X₄ is selected from the group consisting of alanine, serine, and threonine;

10 (b) forming a reaction mixture including: (i) the expressed polypeptide; (ii) the substantially purified sortase-transamidase enzyme of claim 27; and (iii) a Gram-positive bacterium having a peptidoglycan to which the sortase-transamidase can link the polypeptide; and

15 (c) allowing the sortase-transamidase to catalyze a reaction that cleaves the polypeptide within the LPX₃X₄G motif of the sorting signal and covalently cross-links the amino-terminal portion of the cleaved polypeptide to the peptidoglycan to display the polypeptide on the surface of the Gram-positive bacterium.

59. A method for displaying a polypeptide on the surface of a Gram-positive bacterium comprising the steps of:

5 (a) expressing a polypeptide having a sorting signal at its carboxy-terminal end, the sorting signal having: (1) a motif of LPX₃X₄G therein; (2) a substantially hydrophobic domain of at least 31 amino acids carboxyl to the motif; and (3) a charged tail region with at least two positively charged residues carboxyl to the substantially hydrophobic domain, at least one of the two positively charged residues being arginine, the two positively charged residues being located at residues 31-33 from the motif, wherein X₃ is any of the twenty naturally-occurring L-amino acids and X₄ is selected from the group consisting of alanine, serine, and threonine;

10 (b) forming a reaction mixture including: (i) the expressed polypeptide; (ii) the substantially purified sortase-transamidase enzyme of claim 28; and (iii) a Gram-positive bacterium having a peptidoglycan to which the sortase-transamidase can link the polypeptide; and

15 (c) allowing the sortase-transamidase to catalyze a reaction that cleaves the polypeptide within the LPX₃X₄G motif of the sorting signal and covalently cross-links the amino-terminal portion of the cleaved polypeptide to the peptidoglycan to display the polypeptide on the surface of the Gram-positive bacterium.

60. A method for displaying a polypeptide on the surface of a Gram-positive bacterium comprising the steps of:

5 (a) expressing a polypeptide having a sorting signal at its carboxy-terminal end, the sorting signal having: (1) a motif of LPX₃X₄G therein; (2) a substantially hydrophobic domain of at least 31 amino acids carboxyl to the motif; and (3) a charged tail region with at least two positively charged residues carboxyl to the substantially hydrophobic domain, at least one of the two positively charged residues being arginine, the two positively charged residues being located at residues 31-33 from the motif, wherein X₃ is any of the twenty naturally-occurring L-amino acids and X₄ is selected from the group consisting of alanine, serine, and threonine;

10 (b) forming a reaction mixture including: (i) the expressed polypeptide; (ii) the substantially purified sortase-transamidase enzyme of claim 29; and (iii) a Gram-positive bacterium having a peptidoglycan to which the sortase-transamidase can link the polypeptide; and

15 (c) allowing the sortase-transamidase to catalyze a reaction that cleaves the polypeptide within the LPX₃X₄G motif of the sorting signal and covalently cross-links the amino-terminal portion of the cleaved polypeptide to the peptidoglycan to display the polypeptide on the surface of the Gram-positive bacterium.

50b C1 61. A method for displaying a polypeptide on the surface of a Gram-positive bacterium comprising the steps of:

(a) cloning a nucleic acid segment encoding a chimeric protein into a Gram-positive bacterium to generate a cloned chimeric protein including therein a carboxyl-terminal sorting signal, the chimeric protein including the polypeptide to be displayed, the sorting signal having: (1) a motif of LPX_3X_4G therein; (2) a substantially hydrophobic domain of at least 31 amino acids carboxyl to the motif; and (3) a charged tail region with at least two positively charged residues carboxyl to the substantially hydrophobic domain, at least one of the two positively charged residues being arginine, the two positively charged residues being located at residues 31-33 from the motif, wherein X_3 is any of the twenty naturally-occurring L-amino acids and X_4 is selected from the group consisting of alanine, serine, and threonine;

(b) growing the bacterium into which the nucleic acid segment has been cloned to express the cloned chimeric protein to generate a chimeric protein including therein a carboxyl-terminal sorting signal; and

(c) binding the polypeptide covalently to the cell wall by the enzymatic action of a sortase-transamidase expressed by the Gram-positive bacterium involving cleavage of the chimeric protein within the LPX_3X_4G motif so that the polypeptide is displayed on the surface of the Gram-positive bacterium in such a way that the polypeptide is accessible to a ligand.

62. A polypeptide displayed on the surface of a Gram-positive bacterium by covalent linkage of an amino-acid sequence of LPX_3X_4 derived from cleavage of an LPX_3X_4G motif, wherein X_3 is any of the twenty naturally-occurring L-amino acids and X_4 is selected from the group consisting of alanine, serine, and threonine, the polypeptide being displayed on the surface of the Gram-positive bacterium in such a way that the polypeptide is accessible to a ligand.

63. A covalent complex comprising:

- (a) the polypeptide of claim 62; and
- (b) an antigen or hapten covalently cross-linked to the polypeptide.

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64. The covalent complex of claim 63 wherein an antigen is covalently cross-linked to the polypeptide.

65. The covalent complex of claim 63 wherein a hapten is covalently cross-linked to the peptide.

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66. A method for vaccination of an animal comprising the step of immunizing the animal with the displayed polypeptide of claim 62 to generate an immune response against the displayed polypeptide.

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67. A method for vaccination of an animal comprising the step of immunizing the animal with the covalent complex of claim 63 to generate an immune response against the antigen or hapten of the covalent complex.

5ub C1 68. A method for screening for expression of a cloned polypeptide comprising the steps of:

(a) expressing a cloned polypeptide as a chimeric protein having a sorting signal at its carboxy-terminal end, the sorting signal having: (1) a motif of LPX_3X_4G therein; (2) a substantially hydrophobic domain of at least 31 amino acids carboxyl to the motif; and (3) a charged tail region with at least two positively charged residues carboxyl to the substantially hydrophobic domain, at least one of the two positively charged residues being arginine, the two positively charged residues being located at residues 31-33 from the motif, wherein X_3 is any of the twenty naturally-occurring L-amino acids and X_4 is selected from the group consisting of alanine, serine, and threonine;

(b) forming a reaction mixture including: (i) the expressed chimeric protein; the substantially purified sortase-transamidase enzyme of claim 1; and (iii) a Gram-positive bacterium having a peptidoglycan to which the sortase-transamidase can link the polypeptide through the sorting signal;

(c) binding the chimeric protein covalently to the cell wall by the enzymatic action of a sortase-transamidase expressed by the Gram-positive bacterium involving cleavage of the chimeric protein within the LPX_3X_4G motif so that the polypeptide is displayed on the surface of the Gram-positive bacterium in such a way that the polypeptide is accessible to a ligand; and

(d) reacting the displayed polypeptide with a labeled specific binding partner to screen the chimeric protein for reactivity with the labeled specific binding partner.

506 47 69. A method for screening for expression of a cloned polypeptide comprising the steps of:

(a) expressing a cloned polypeptide as a chimeric protein having a sorting signal at its carboxy-terminal end, the sorting signal having: (1) a motif of LPX_3X_4G therein; (2) a substantially hydrophobic domain of at least 31 amino acids carboxyl to the motif; and (3) a charged tail region with at least two positively charged residues carboxyl to the substantially hydrophobic domain, at least one of the two positively charged residues being arginine, the two positively charged residues being located at residues 31-33 from the motif, wherein X_3 is any of the twenty naturally-occurring L-amino acids and X_4 is selected from the group consisting of alanine, serine, and threonine;

(b) forming a reaction mixture including: (i) the expressed chimeric protein; (ii) the substantially purified sortase-transamidase enzyme of claim 3; and (iii) a Gram-positive bacterium having a peptidoglycan to which the sortase-transamidase can link the polypeptide through the sorting signal;

(c) binding the chimeric protein covalently to the cell wall by the enzymatic action of a sortase-transamidase expressed by the Gram-positive bacterium involving cleavage of the chimeric protein within the LPX_3X_4G motif so that the polypeptide is displayed on the surface of the Gram-positive bacterium in such a way that the polypeptide is accessible to a ligand; and

(d) reacting the displayed polypeptide with a labeled specific binding partner to screen the chimeric protein for reactivity with the labeled specific binding partner.

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70. A method for screening for expression of a cloned polypeptide comprising the steps of:

(a) expressing a cloned polypeptide as a chimeric protein having a sorting signal at its carboxy-terminal end, the sorting signal having: (1) a motif of LPX_3X_4G therein; (2) a substantially hydrophobic domain of at least 31 amino acids carboxyl to the motif; and (3) a charged tail region with at least two positively charged residues carboxyl to the substantially hydrophobic domain, at least one of the two positively charged residues being arginine, the two positively charged residues being located at residues 31-33 from the motif, wherein X_3 is any of the twenty naturally-occurring L-amino acids and X_4 is selected from the group consisting of alanine, serine, and threonine;

(b) forming a reaction mixture including: (i) the expressed chimeric protein; (ii) the substantially purified sortase-transamidase enzyme of claim 26; and (iii) a Gram-positive bacterium having a peptidoglycan to which the sortase-transamidase can link the polypeptide through the sorting signal;

(c) binding the chimeric protein covalently to the cell wall by the enzymatic action of a sortase-transamidase expressed by the Gram-positive bacterium involving cleavage of the chimeric protein within the LPX_3X_4G motif so that the polypeptide is displayed on the surface of the Gram-positive bacterium in such a way that the polypeptide is accessible to a ligand; and

(d) reacting the displayed polypeptide with a labeled specific binding partner to screen the chimeric protein for reactivity with the labeled specific binding partner.

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71. A method for screening for expression of a cloned polypeptide comprising the steps of:

(a) expressing a cloned polypeptide as a chimeric protein having a sorting signal at its carboxy-terminal end, the sorting signal having: (1) a motif of LPX_3X_4G therein; (2) a substantially hydrophobic domain of at least 31 amino acids carboxyl to the motif; and (3) a charged tail region with at least two positively charged residues carboxyl to the substantially hydrophobic domain, at least one of the two positively charged residues being arginine, the two positively charged residues being located at residues 31-33 from the motif, wherein X_3 is any of the twenty naturally-occurring L-amino acids and X_4 is selected from the group consisting of alanine, serine, and threonine;

(b) forming a reaction mixture including: (i) the expressed chimeric protein; (ii) the substantially purified sortase-transamidase enzyme of claim 27; and (iii) a Gram-positive bacterium having a peptidoglycan to which the sortase-transamidase can link the polypeptide through the sorting signal;

(c) binding the chimeric protein covalently to the cell wall by the enzymatic action of a sortase-transamidase expressed by the Gram-positive bacterium involving cleavage of the chimeric protein within the LPX_3X_4G motif so that the polypeptide is displayed on the surface of the Gram-positive bacterium in such a way that the polypeptide is accessible to a ligand; and

(d) reacting the displayed polypeptide with a labeled specific binding partner to screen the chimeric protein for reactivity with the labeled specific binding partner.

506 C1 72. A method for screening for expression of a cloned polypeptide comprising the steps of:

(a) expressing a cloned polypeptide as a chimeric protein having a sorting signal at its carboxy-terminal end, the sorting signal having: (1) a motif of LPX_3X_4G therein; (2) a substantially hydrophobic domain of at least 31 amino acids carboxyl to the motif; and (3) a charged tail region with at least two positively charged residues carboxyl to the substantially hydrophobic domain, at least one of the two positively charged residues being arginine, the two positively charged residues being located at residues 31-33 from the motif, wherein X_3 is any of the twenty naturally-occurring L-amino acids and X_4 is selected from the group consisting of alanine, serine, and threonine;

(b) forming a reaction mixture including: (i) the expressed chimeric protein; (ii) the substantially purified sortase-transamidase enzyme of claim 28; and (iii) a Gram-positive bacterium having a peptidoglycan to which the sortase-transamidase can link the polypeptide through the sorting signal;

(c) binding the chimeric protein covalently to the cell wall by the enzymatic action of a sortase-transamidase expressed by the Gram-positive bacterium involving cleavage of the chimeric protein within the LPX_3X_4G motif so that the polypeptide is displayed on the surface of the Gram-positive bacterium in such a way that the polypeptide is accessible to a ligand; and

(d) reacting the displayed polypeptide with a labeled specific binding partner to screen the chimeric protein for reactivity with the labeled specific binding partner.

506 17
73. A method for screening for expression of a cloned polypeptide comprising the steps of:

(a) expressing a cloned polypeptide as a chimeric protein having a sorting signal at its carboxy-terminal end, the sorting signal having: (1) a motif of LPX_3X_4G therein; (2) a substantially hydrophobic domain of at least 31 amino acids carboxyl to the motif; and (3) a charged tail region with at least two positively charged residues carboxyl to the substantially hydrophobic domain, at least one of the two positively charged residues being arginine, the two positively charged residues being located at residues 31-33 from the motif, wherein X_3 is any of the twenty naturally-occurring L-amino acids and X_4 is selected from the group consisting of alanine, serine, and threonine;

(b) forming a reaction mixture including: (i) the expressed chimeric protein; (ii) the substantially purified sortase-transamidase enzyme of claim 29; and (iii) a Gram-positive bacterium having a peptidoglycan to which the sortase-transamidase can link the polypeptide through the sorting signal;

(c) binding the chimeric protein covalently to the cell wall by the enzymatic action of a sortase-transamidase expressed by the Gram-positive bacterium involving cleavage of the chimeric protein within the LPX_3X_4G motif so that the polypeptide is displayed on the surface of the Gram-positive bacterium in such a way that the polypeptide is accessible to a ligand; and

(d) reacting the displayed polypeptide with a labeled specific binding partner to screen the chimeric protein for reactivity with the labeled specific binding partner.

5064 74. A method for screening for expression of a cloned polypeptide comprising the steps of:

(a) cloning a nucleic acid segment encoding a chimeric protein into a Gram-positive bacterium to generate a cloned chimeric protein including therein a carboxyl-terminal sorting signal, the chimeric protein including the polypeptide whose expression is to be screened, the sorting signal having: (1) a motif of LPX_3X_4G therein; (2) a substantially hydrophobic domain of at least 31 amino acids carboxyl to the motif; and (3) a charged tail region with at least two positively charged residues carboxyl to the substantially hydrophobic domain, at least one of the two positively charged residues being arginine, the two positively charged residues being located at residues 31-33 from the motif, wherein X_3 is any of the twenty naturally-occurring L-amino acids and X_4 is selected from the group consisting of alanine, serine, and threonine;

(b) growing the bacterium into which the nucleic acid segment has been cloned to express the cloned chimeric protein to generate a chimeric protein including therein a carboxyl-terminal sorting signal;

(c) binding the polypeptide covalently to the cell wall by the enzymatic action of a sortase-transamidase expressed by the Gram-positive bacterium involving cleavage of the chimeric protein within the LPX_3X_4G motif so that the polypeptide is displayed on the surface of the Gram-positive bacterium in such a way that the polypeptide is accessible to a ligand; and

(d) reacting the displayed polypeptide with a labeled specific binding partner to screen the chimeric protein for reactivity with the labeled specific binding partner.

50647
75. A method for the diagnosis or treatment of a bacterial infection caused by a Gram-positive bacterium comprising the steps of:

(a) conjugating an antibiotic or a detection reagent to a protein including therein a carboxyl-terminal sorting signal to produce a conjugate, the carboxyl-terminal sorting signal having: (1) a motif of LPX₃X₄G therein; (2) a substantially hydrophobic domain of at least 31 amino acids carboxyl to the motif; and (3) a charged tail region with at least two positively charged residues carboxyl to the substantially hydrophobic domain, at least one of the two positively charged residues being arginine, the two positively charged residues being located at residues 31-33 from the motif, wherein X₃ is any of the twenty naturally-occurring L-amino acids and X₄ is selected from the group consisting of alanine, serine, and threonine; and

(b) introducing the conjugate to an organism infected with a Gram-positive bacterium in order to cause the conjugate to be sorted and covalently cross-linked to the cell walls of the bacterium in order to treat or diagnose the infection.

76. The method of claim 75 wherein an antibiotic is conjugated to the protein.

77. The method of claim 76 wherein the antibiotic is selected from the group consisting of a penicillin, ampicillin, vancomycin, gentamicin, streptomycin, a cephalosporin, amikacin, kanamycin, neomycin, paromomycin, tobramycin, ciprofloxacin, clindamycin, rifampin, chloramphenicol, norfloxacin, and a derivative of these antibiotics.

78. The method of claim 75 wherein a detection reagent is conjugated to the protein.

sub 47

79. A conjugate comprising an antibiotic or a detection reagent covalently conjugated to a protein including therein a carboxyl-terminal sorting signal to produce a conjugate, the carboxyl-terminal sorting signal having: (1) a motif of LPX₃X₄G therein; (2) a substantially hydrophobic domain of at least 31 amino acids carboxyl to the motif; and (3) a charged tail region with at least two positively charged residues carboxyl to the substantially hydrophobic domain, at least one of the two positively charged residues being arginine, the two positively charged residues being located at residues 31-33 from the motif, wherein X₃ is any of the twenty naturally-occurring L-amino acids and X₄ is selected from the group consisting of alanine, serine, and threonine.

80. The conjugate of claim 79 wherein an antibiotic is conjugated to the protein.

81. The conjugate of claim 80 wherein the antibiotic is selected from the group consisting of a penicillin, ampicillin, vancomycin, gentamicin, streptomycin, a cephalosporin, amikacin, kanamycin, neomycin, paromomycin, tobramycin, ciprofloxacin, clindamycin, rifampin, chloramphenicol, norfloxacin, and a derivative of these antibiotics.

82. The conjugate of claim 79 wherein a detection reagent is conjugated to the protein.

83. A composition comprising:
(a) the conjugate of claim 79; and
(b) a pharmaceutically acceptable carrier.

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84. A substantially purified protein having at least about 30% sequence similarity with the amino acid sequences of at least one of *S. pyogenes* (SEQ ID NO: 4), *A. naeslundii* (SEQ. ID NO. 5), *E. faecalis* (SEQ. ID NO. 6), *S. mutans* (SEQ. ID NO. 7) *S. pneumoniae* (SEQ. ID NO. 34, SEQ. ID NO. 35, or SEQ ID NO. 36) or *B. subtilis* (SEQ. ID NO. 8) and having sortase-transamidase activity.

85. The substantially purified protein of claim 84 wherein the sequence similarity with the amino acid sequences of at least one of *S. pyogenes* (SEQ ID NO: 4), *A. naeslundii* (SEQ. ID NO. 5), *E. faecalis* (SEQ. ID NO. 6), *S. mutans* (SEQ. ID NO. 7) *S. pneumoniae* (SEQ. ID NO. 34, SEQ. ID NO. 35, or SEQ ID NO. 36) or *B. subtilis* (SEQ. ID NO. 8) is at least about 40%.

86. The substantially purified protein of claim 85 wherein the sequence similarity with the amino acid sequences of at least one of *S. pyogenes* (SEQ ID NO: 4), *A. naeslundii* (SEQ. ID NO. 5), *E. faecalis* (SEQ. ID NO. 6), *S. mutans* (SEQ. ID NO. 7) *S. pneumoniae* (SEQ. ID NO. 34, SEQ. ID NO. 35, or SEQ ID NO. 36) or *B. subtilis* (SEQ. ID NO. 8) is at least about 50%.

87. A substantially purified protein having at least about 18% sequence identity with the amino acid sequences of at least one of *S. pyogenes* (SEQ ID NO: 4), *A. naeslundii* (SEQ. ID NO. 5), *E. faecalis* (SEQ. ID NO. 6), *S. mutans* (SEQ. ID NO. 7) *S. pneumoniae* (SEQ. ID NO. 34, SEQ. ID NO. 35, or SEQ ID NO. 36) or *B. subtilis* (SEQ. ID NO. 8) and having sortase-transamidase activity.

88. The substantially purified protein of claim 84 wherein the sequence identity with the amino acid sequences of at least one of *S. pyogenes* (SEQ ID NO: 4), *A. naeslundii* (SEQ. ID NO. 5), *E. faecalis* (SEQ. ID NO. 6), *S. mutans* (SEQ. ID NO. 7) *S. pneumoniae*

(SEQ. ID NO. 34, SEQ. ID NO. 35, or SEQ ID NO. 36) or *B. subtilis* (SEQ. ID NO. 8) is at least about 20%.

5 89. The substantially purified protein of claim 85 wherein the sequence identity with the amino acid sequences of at least one of *S. pyogenes* (SEQ ID NO: 4), *A. naeshlundii* (SEQ. ID NO. 5), *E. faecalis* (SEQ. ID NO. 6), *S. mutans* (SEQ. ID NO. 7) *S. pneumoniae* (SEQ. ID NO. 34, SEQ. ID NO. 35, or SEQ ID NO. 36) or *B. subtilis* (SEQ. ID NO. 8) is at least about 30%.

10 90. A nucleic acid sequence encoding the substantially purified protein of claim 84.

15 91. A vector comprising the nucleic acid sequence of claim 90 operatively linked to at least one control sequence that controls the expression or regulation of the nucleic acid sequence.

20 92. A host cell transfected with the vector of claim 91.

25 93. A method for producing a substantially purified protein having sortase-transamidase activity comprising the steps of:

(a) culturing the host cell of claim 92 under conditions in which the host cell expresses the protein having sortase-transamidase activity; and

(b) purifying the expressed protein to produce substantially purified protein having sortase-transamidase activity.

30 94. A nucleic acid sequence encoding the substantially purified protein of claim

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sub 4) 95. A vector comprising the nucleic acid sequence of claim 94 operatively linked to at least one control sequence that controls the expression or regulation of the nucleic acid sequence.

5 96. A host cell transfected with the vector of claim 95.

97. A method for producing a substantially purified protein having sortase-transamidase activity comprising the steps of:

10 (a) culturing the host cell of claim 96 under conditions in which the host cell expresses the protein having sortase-transamidase activity; and

(b) purifying the expressed protein to produce substantially purified protein having sortase-transamidase activity.